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EP 0 328 111 A2

㉚ Cancer cell metastasis inhibitors.

㉛ Sugar lactams of formula (I) such as N-(3-phenylpropyl)-1-deoxynojirimycin, 1-deoxynojirimycin, D-glucaro- δ -lactam, 6-O-triphenylmethyl-D-gluco- δ -lactam, etc. markedly inhibit metastasis of cancer cells. Sugar lactam derivatives of formula (I') are novel compounds.

CANCER CELL METASTASIS INHIBITORS AND NOVEL COMPOUNDS

BACKGROUND OF THE INVENTION5 Field of the Invention

The present invention relates to compositions capable of markedly inhibiting formation of metastatic lesion of cancer cells and also relates to novel sugar lactams having such an activity.

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Description of the Prior Art

Main trends in known anticancer agents are drugs to kill malignant cells via cytotoxicity possessed by substances or via human immune system. However, these drugs are yet unsatisfactory for treatment of cancer. To solid cancer, treatment by surgical operation or radiotherapy has been applied and chances of success are greatly improved to the extent that these treatments could eliminate primary cancer. However, the most serious factor for affecting prognosis of the patient with cancer to whom these treatments have been applied is cancer metastasis. Accordingly, it is expected that the effectiveness of the existing various therapies could be further enhanced by inhibiting metastasis of cancer cells. However, there are few substances showing inhibition of metastasis as their main activity and none of them has been used in the clinical field.

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SUMMARY OF THE INVENTION

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The present invention is based on the finding substances which can markedly inhibit metastasis of cancer cells and can be used for effective and proper treatment of cancer and aims at providing cancer cell metastasis inhibitors comprising these substances as the effective ingredient.

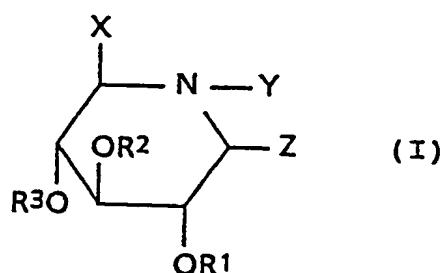
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Metastasis of cancer takes a process of (a) release of cancer cells from primary cancer, (b) seeding into vessel and (c) reaching the target tissue via adherence and extravasation in capillary blood vessel to commence growth.

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The present inventors have used the experimental system for evaluation of this cancer metastasis inhibiting activity and have found that compounds represented by general formula (I) described below possess an extremely excellent cancer metastasis inhibiting activity; the present inventors have also prepared compounds represented by general formula (I') as novel compounds exhibiting the activity described above and, thus have accomplished the present invention.

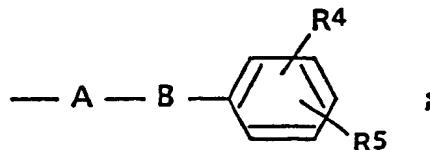
General formula (I)



In formula (I):

a)
 X : -CH₂OH;
 Y : hydrogen atom or

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A : hydrocarbon group having 3 to 5 carbon atoms which may or may not contain a double bond;

B : bond or, oxygen atom or sulfur atom;

R⁴, R⁵ : independently hydrogen atom, halogen atom or

15 a lower alkyl group;

Z : hydrogen atom;

R¹, R², R³ : hydrogen atom

b)

X : -COOR⁶ (R⁶ : hydrogen atom or an alkyl group having 1 to 8 carbon atoms, pivaloyloxymethyl group or (5-methyl-2-oxo 1,3-dioxole-4-yl)methyl group);

20 Y : hydrogen atom;

Z : = O;

R¹, R², R³ : hydrogen atom

c)

25 X : -CH₂OR⁷

Y : hydrogen atom;

Z : = O;

R¹, R², R⁷ : independently hydrogen atom or WCO-, wherein W represents an alkyl, a substituted alkyl, an aryl, a substituted aryl, an aralkyl or a substituted aralkyl group (provided that all are not hydrogen atom simultaneously);

30 R³ : hydrogen atom;

or

d)

X : -CH₂OR⁸;

35 Y : hydrogen atom;

Z : = O;

R¹, R³ : independently hydrogen atom or an acyl group;

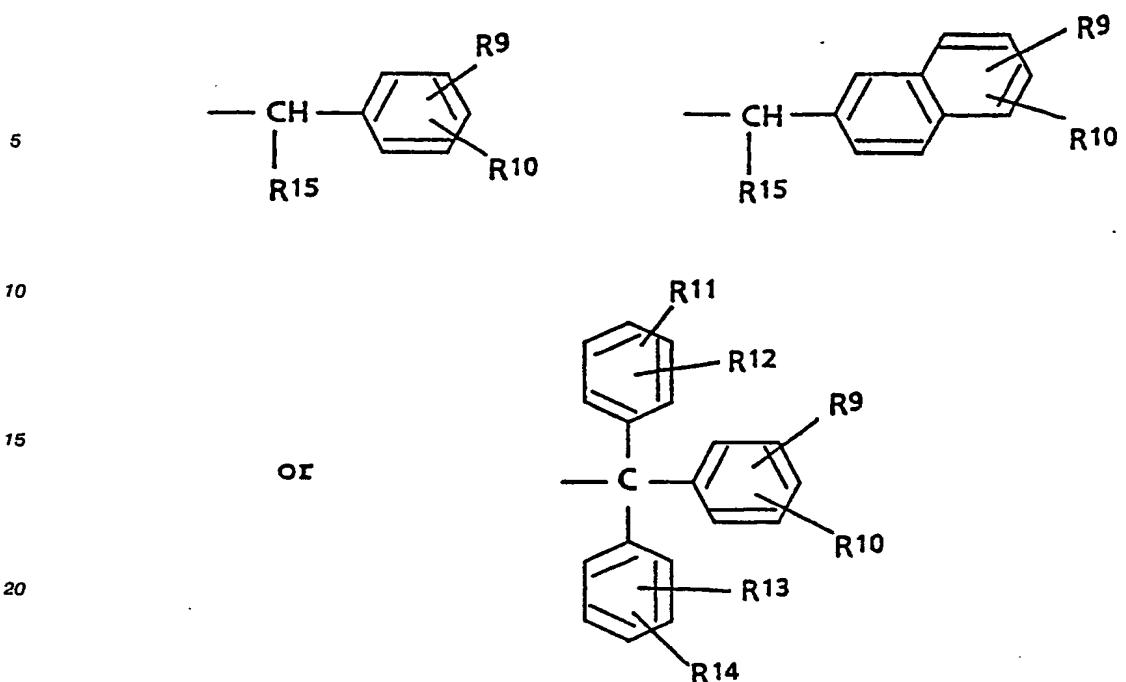
R², R⁸ : independently hydrogen atom,

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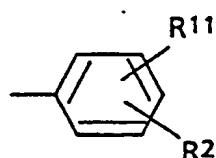
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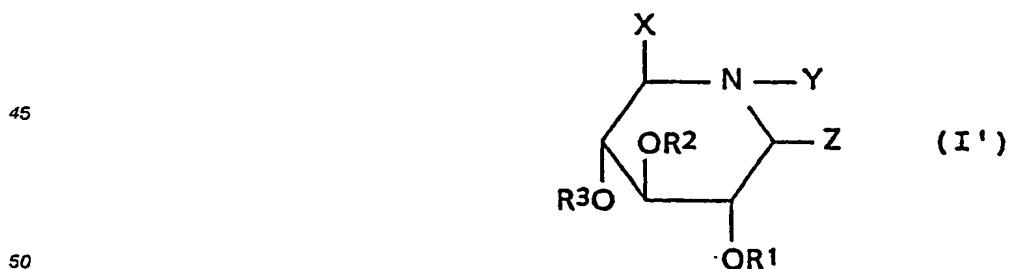
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R⁹ - R¹⁴: independently hydrogen atom, a halogen atom, a lower alkyl, a lower alkoxy, a halogenomethyl, carboxyl, a carboxyalkyl, a carboalkoxyalkyl, phenyl or nitro group
 R¹⁵ : a lower alkyl group or



40 General formula (I')



In formula (I'):

a)

55 X : -CH₂OR⁷;
 Y : hydrogen atom;
 Z : = O;
 R¹, R², R⁷: independently hydrogen atom or WCO-, wherein W represents an alkyl, a substituted alkyl, an aryl, a substituted aryl, an aralkyl or a substituted aralkyl group (provided that all are not hydrogen atom

simultaneously);

R³ : hydrogen atom;

or,

b)

5 X : -CH₂OR⁸;

Y : hydrogen atom;

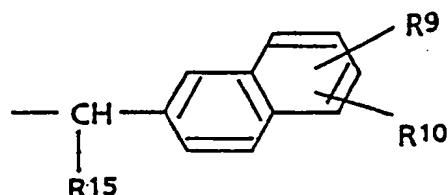
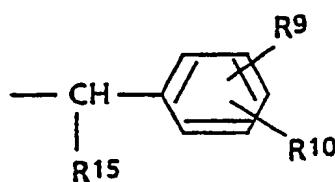
Z : = O

R¹, R³ : hydrogen atom or an acyl group;

R², R⁸ : independently hydrogen atom,

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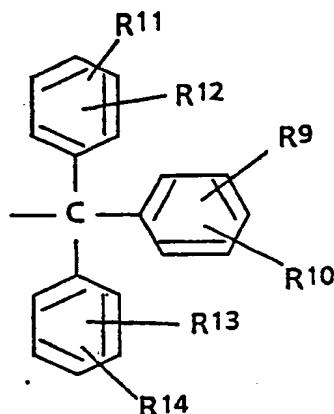


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or

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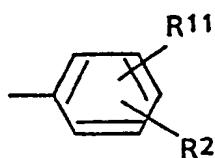


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R⁹ - R¹⁴ : independently hydrogen atom, a halogen atom, a lower alkyl, a lower alkoxy, a halogenomethyl, carboxyl, a carboxyalkyl, a carboalkoxyalkyl, phenyl or nitro group

R¹⁵ : a lower alkyl or

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The compounds represented by general formula (I') of the present invention can be prepared by the following processes.

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(1) Production of compounds represented by general formula (I') wherein X, Y, Z, R¹, R² and R³ are described in a) (Compound I'a):

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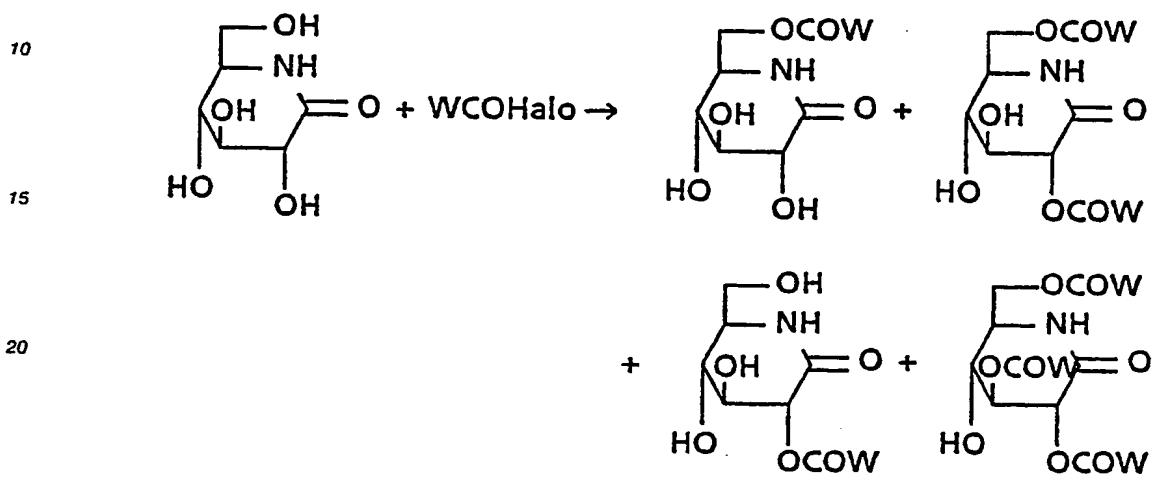
D-Glucosidase which is a raw material for synthesis of the compounds can easily be obtained by oxidizing nojirimycin (5-amino-5-deoxy-D-glucopyranose) (Japanese Published Examined Patent Application No. 760/1968, Tetrahedron, 24, 2125 - 2144, 1968) that is a metabolite of Actinomycetes (Scientific Reports of Meiji Seika Kaisha, No. 13, 80-84, 1973, Japanese Published Examined Patent Application No.

28375/1970).

The sugar lactam derivatives represented by general formula (Ia) of the present invention can be synthesized by the following steps, using this D-gluco- δ -lactam as the raw material.

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Process A:



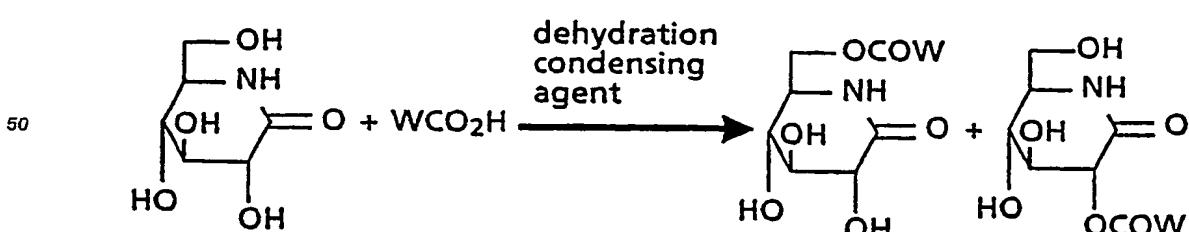
wherein W has the same significance as described above and Halo represents a halogen atom.

The reaction can be performed by first reacting a carboxylic acid component WCOOH (wherein W has the same significance as defined above) with an acid halide synthesis reagent such as thionyl chloride, thionyl bromide, phosphorus oxychloride, etc. in an inert solvent such as methylene chloride, 1, 2-dichloroethane, etc. to give the acid halide (WCOHalo). The reaction is carried out generally at a reaction temperature ranging from 5 to 100 °C and the reaction time is 1 to 24 hours. The thus obtained acid halide is used for the next condensation step, generally without isolating the acid halide, merely by distilling off the reaction solvent under reduced pressure.

35 The obtained acid halide is dissolved in an inert solvent (N,N-dimethylformamide, halogenated hydrocarbon solvents, acetonitrile, tetrahydrofuran, dioxane, etc.) to form the solution, or, in the absence of any solvent, added to a solution or suspension of D-gluco- δ -lactam in an inert solvent (N,N-dimethylformamide, dioxane, pyridine, halogenated hydrocarbon solvents, etc.) in the presence of an inorganic base such as potassium carbonate, sodium hydrogencarbonate, etc. or an organic base such as triethylamine, pyridine, etc., followed by condensation. The reaction is carried out generally at a reaction temperature ranging from -15 to 80 °C; the reaction time is 1 to 24 hours.

Process B:

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The reaction is carried out by reacting with a carboxylic acid component WCOOH (wherein W has the same significance as described above) and a dehydrating agent, for example, 1, 3-didyclohexylcarbodiimide, diethylcyanophosphate, diphenyl phosphorylazide, p-toluenesulfonyl chloride, triphenylphosphine

diethylazodicarboxylate, 1,1'-carbonyldiimidazole, etc., together, in an inert solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, dimethyl sulfoxide, pyridine, etc., if necessary and desired, in the presence of an organic base such as triethylamine, pyridine, diisopropylethylamine, etc. The reaction is carried out at a reaction temperature generally ranging from 5 to 100 °C and for a reaction time of 1 to 48 hours.

The compound of formula (I'a) thus obtained is collected from the reaction mixture in a conventional manner. The compound (I'a) is isolated, for example, by extraction with an organic solvent or water, precipitation, crystallization or a variety means of chromatography, as a pure substance.

Specific examples of Compound (I'a) include the following compounds:

10 2-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl)-D-gluco- δ -lactam, 6-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl)-D-gluco- δ -lactam, 2,6-di-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl)-D-gluco- δ -lactam, 2-O-(3-benzoylhydratropoyl)-D-gluco- δ -lactam, 6-O-(3-benzoylhydratropoyl)-D-gluco- δ -lactam, 2,6-di-O-(3-benzoylhydratropoyl)-D-gluco- δ -lactam, 2-O-(6-methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam, 6-O-(6-methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam, 2,6-di-O-(6-methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam, 6-O-(N-(2,3-xylyl)anthranyloyl)-D-gluco- δ -lactam, 6-O-(acetylsalicyloyl)-D-gluco- δ -lactam, 2,6-di-O-(acetylsalicyloyl)-D-gluco- δ -lactam, 2,3,6-tri-O-(acetylsalicyloyl)-D-gluco- δ -lactam, 6-O-diphenylacetyl-D-gluco- δ -lactam, 6-O-triphenylacetyl-D-gluco- δ -lactam.

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(2) Production of compound shown by general formula (I') wherein X, Y, Z, R¹, R² and R³ are described in b) (Compound I'b):

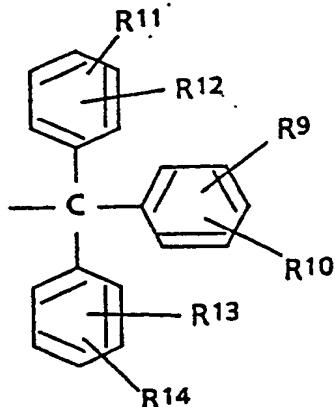
Among Compound I'b, Compound (I-b-A) wherein R² and R³ take:

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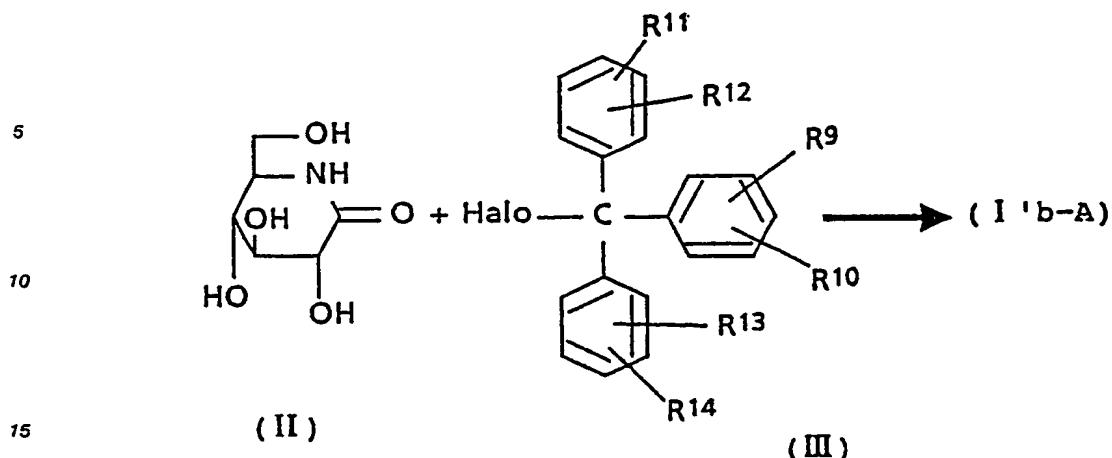


wherein R⁹ through R¹⁴ have the same significance as described above, can be produced by the following reaction scheme:

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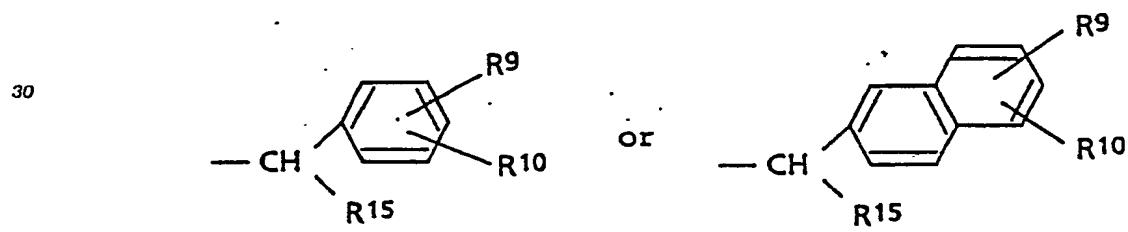
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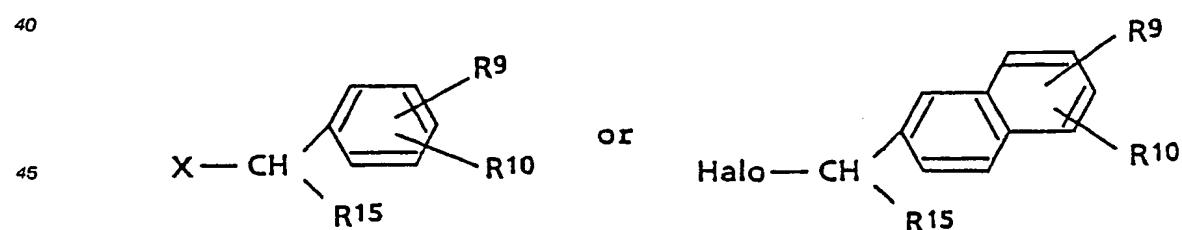
wherein Halo and R⁹ through R¹⁴ have the same significance as described above.

20 The reaction can be carried out by reaction D-gluco- δ -lactam (II) with activated trityl compound (III) in an inert polar solvent such as N,N-dimethylformamide, N,N-dimethylacetamide, dimethylsulfoxide, pyridine, etc., in the presence of an inorganic base such as potassium carbonate, sodium carbonate, sodium hydride, barium oxide, sodium hydrogencarbonate, etc., or an organic base such as triethylamine, pyridine, disopropylethylamine, dicyclohexylamine, 1,5-diazabicyclo (4.3.0)non-5-ene, etc., generally at 5 to 100 °C for 1 to 48 hours.

25 Among Compound I' b, Compound (I' b-B) wherein R² and R⁸ represent:

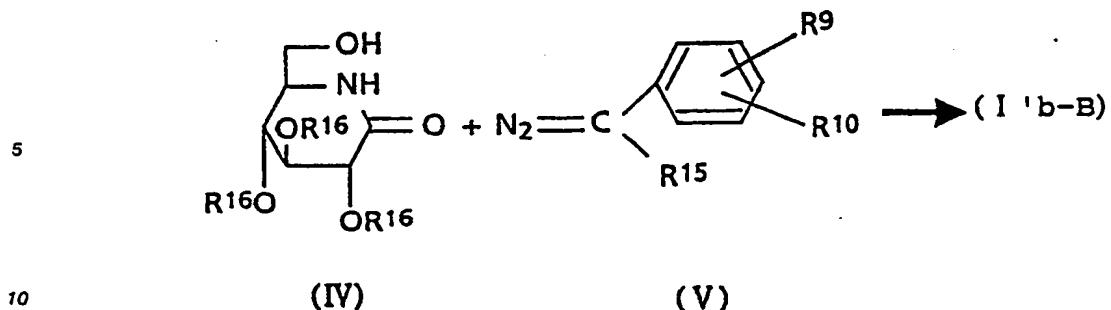


wherein R⁹, R¹⁰ and R¹⁵ have the same significance as described above, can be produced by reacting the following compound:



50 wherein Hal, R⁹, R¹⁰, and R¹⁵ have the same significances as described above, with D-gluco- δ -lactam (II), in a manner similar to the production of Compound (I' b-A) described above.

However, the reaction temperature is generally as high as, for example, 50 to 200 °C, or, selectivity to the 3- or 6-position of D-gluco- δ -lactam is low. Therefore, the reaction shown below using diazo compounds is simpler.



wherein R¹⁶ represents a hydrogen atom or an acyl protecting group and R⁹, R¹⁰ and R¹⁵ have the same significances as described above.

15 This reaction is carried out in an inert solvent such as methylene chloride, 1,2 dichloroethane, tetrahydrofuran, dioxane, acetone, ethyl acetate, toluene, acetonitrile, N,N-dimethylformamide, etc. or a solvent mixture thereof, generally in the presence of an acid catalyst. As the acid catalyst used, mention may be made of protonic acids such as tetrafluoroboric acid, perchloric acid, trifluoromethanesulfonic acid, etc., or tetrafluoroboric acid, tetrachloric tin, ferric chloride, antimony pentafluoride, zinc chloride, etc.

20 Compound (I' b-B) can be obtained at a reaction temperature of from -60 to + 50 °C for a reaction time of 1 to 24 hours.

Specific examples of Compound I' b include the following compounds:

25 3-O-trityl-D-glucosidolactam, 6-O-trityl-D-glucosidolactam, 3,6 di-O-trityl-D-glucosidolactam, 6-O-(3,5-ditri fluoromethyl-trityl)-D-glucosidolactam, 6-O-(3,3'-ditri fluoromethyltrityl)-D-glucosidolactam, 6-O-(4-tertbutyl-trityl)-D-glucosidolactam, 6-O-(4-carboxytrityl)-D-glucosidolactam, 6-O-benzhydryl-D-glucosidolactam, 6-O-(3,3'-ditri fluoromethylbenzhydryl)-D-glucosidolactam, 6-O-(1-β-naphthylethyl)-D-glucosidolactam, 6-O-(p-phenylbenzhydryl)-D-glucosidolactam, 6-O-(1-phenylethyl)-D-glucosidolactam, 6-O-(2-chloro-5-nitrobenzhydryl)-D-glucosidolactam, 6-O-(3,4-dichlorobenzhydryl)-D-glucosidolactam, 6-O-(4,4'-dichlorobenzhydryl)-D-glucosidolactam, 6-O-(2,4-difluorobenzhydryl)-D-glucosidolactam, 6-O-(4-nitrobenzhydryl)-D-glucosidolactam, 6-O-(1-p-phenoxyethyl)-D-glucosidolactam, 6-O-(3,4-dimethylbenzhydryl)-D-glucosidolactam, 6-O-(m-1-tertbutyloxycarbonylethyl)-benzhydryl)-D-glucosidolactam.

35 (3) Production of compound shown by general formula (I) wherein X, Y, Z, R¹, R² and R³ are described in a) (Compound Ia):

40 1-Deoxynojirimycin which is a raw material of Compound Ia is a known compound obtained by reduction of nojirimycin (Tetrahedron, 24, 2125 (1968)). Further N-substituted-1-deoxynojirimycins can be obtained by reacting 1-deoxynojirimycin as a raw material with an alkyl halide in a polar solvent such as dimethylformamide (DMF) in the presence of a base such as sodium hydrogencarbonate and isolating the product from the reaction solution (cf. Japanese Published Unexamined Patent Application Nos. 9051/1980 and 47655/1980).

45 Specific examples of Compound Ia include the following compounds:

45 N-(3-phenylpropyl)-1-deoxynojirimycin, N-(4-phenylbutyl)-1-deoxynojirimycin, N-(3-phenylbutyl)-1-deoxynojirimycin, N-(5-phenylpentyl)-1-deoxynojirimycin, N-(3-phenyl-2-propenyl)-1-deoxynojirimycin, N-(4-phenyl-3-butenyl)-1-deoxynojirimycin, N-(3-methyl-3-phenyl-2-propenyl)-1-deoxynojirimycin, N-(3-methylphenyl-2-propenyl)-1-deoxynojirimycin, N-(3-p-methylphenyl-2-propenyl)-1-deoxynojirimycin, N-(3-o-chlorophenyl-2-propenyl)-1-deoxynojirimycin, N-(3-p-chlorophenyl-3-methyl-2-propenyl)-1-deoxynojirimycin, N-(3-p-methylphenyl-3-methyl-2-propenyl)-1-deoxynojirimycin, N-(3-p-bromophenyl-2-propenyl)-1-deoxynojirimycin, N-(2-phenoxyethyl)-1-deoxynojirimycin, N-(3-phenoxypropyl)-1-deoxynojirimycin, N-(3-thiophenoxypropyl)-1-deoxynojirimycin, N-(4-phenoxybutyl)-1-deoxynojirimycin, N-(5-phenoxypentyl)-1-deoxynojirimycin, N-(3-phenoxy-trans-propen-1-yl)-1-deoxynojirimycin, N-(3-phenoxy-trans-2-propenyl)-1-deoxynojirimycin, N-(4-phenoxy-trans-2-butenyl)-1-deoxynojirimycin, N-(3-m-methylphenoxypropyl)-1-deoxynojirimycin, N-(3-o-methylphenoxypropyl)-1-deoxynojirimycin, N-(3-p-methylphenoxypropyl)-1-deoxynojirimycin, N-(3-p-chlorophenoxy-3-methyl-2-propenyl)-1-deoxynojirimycin, N-(3-p-methyl-phenoxy-3-methyl-2-propenyl)-1-deoxynojirimycin, N-(3-p-chlorophenoxy-2-propenyl)-1-deoxynojirimycin, N-(3-m-fluorophenoxy-2-propenyl)-1-deoxynojirimycin, N-(3-p-

bromophenoxy-2-propenyl)-1-deoxynojirimycin, N-(4-p-chlorophenoxy-trans-2-butenyl)-1-deoxynojirimycin.

5 (4) Production of compound shown by general formula (I) wherein X, Y, Z, R¹, R² and R³ are described in b)
 (Compound Ib):

10 Among Compound Ib, the method of producing D-glucaro- δ -lactam has been achieved through chemical or enzymatical oxidation of nojirimycin by the present inventors. That is, nojirimycin is subjected to chemical oxidation or to treatment with glucose oxidase to give the oxidation product of the hydroxy group
 15 at the 1-position thereof (D-gluco- δ -lactam), which is then subjected to catalytic oxidation with air to give D-glucaro- δ -lactam (cf. Scientific Reports of Meiji Seika Kaisha, No. 13, 80-84, 1973 and Japanese Published Examined Patent Application No. 28375/1970).

15 D-Glucaro- δ -lactam alkyl esters can be obtained by reacting D-glucaro- δ -lactam with an alkyl halide or a diazoalkane in methanol, tetrahydrofuran, dioxane, N,N-dimethylformamide, etc. or a solvent mixture thereof (cf. Japanese Published Examined Patent Application Nos. 34589/1981 and 34590/1981).

Specific examples of Compound Ib include the following compounds.

D-glucaro- δ -lactam, D-glucaro- δ -lactam methyl ester, D-glucaro- δ -lactam ethyl ester, D-glucaro- δ -lactam propyl ester, D-glucaro- δ -lactam-n-butyl ester, pivaloyloxymethyl ester, 5-methyl-2-oxo-1,3-dioxole-4-yl methyl ester.

20 Further examples of pharmacologically acceptable salts of the compounds of the present invention include ammonium salts; salts of alkali metals such as sodium, potassium, etc.; salts of alkaline earth metals such as magnesium, calcium, etc.; salts with organic base such as triethylamine, triethanolamine, diethylaminoethylamine, etc.; salts with heterocyclic amines such as piperidine, piperazine, morpholine, etc.; or salts with amino acids such as lysine, etc.

25 Some of these compounds are known to have therapeutic effects for diabetes, (Japanese Published Unexamined Patent Application Nos. 9051/1980, 47655/1980 and 166616/1985). Furthermore, it has been made clear by the present inventors that the compounds falling under general formulae Ia, Ib and Id among the compounds of the present invention are also effective as therapeutic agents for human immunodeficiency virus (HIV) diseases (Japanese Patent Application Nos. 171376/1988, 328387/1987 and 30 326747/1987).

35 The cancer cell metastasis inhibitor of the present invention is administered clinically as oral or parenteral preparations via vein, artery, skin, hypoderm, rectum and muscle or orally. Further by administering against tumor, more potent effects can be expected. Dose may vary depending upon mode of application, preparatory form or age, body weight and condition of patient but generally a dose of 100 to 3000 mg is given once a day or in several portions a day.

40 As parenteral preparations, mention may be made of sterile aqueous or non-aqueous solution or an emulsion. As bases for the non-aqueous solution or emulsion, mention may be made of propylene glycol, polyethylene glycol, glycerin, olive oil, corn oil, ethyl oleate, etc. As oral preparations, mention may be made of capsules, tablets, granules, granulates, powders, etc. In these preparations, starch, lactose, mannitol, ethyl cellulose, sodium carboxymethyl cellulose, etc. are formulated as excipients, and as lubricants, magnesium stearate or calcium stearate is added to the preparations. As binders, gelatin, gum arabic, cellulose esters, polyvinyl pyrrolidone, etc. can be used.

Some examples of the acute toxicities (LD₅₀s) by mice in the claimed compounds are shown in Table 1.

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Table 1

LD ₅₀ Value			
	Compound	Administration Route	LD ₅₀
5	N-(3-phenylpropyl)-1-deoxynojirimycin	i.p.	> 0.5 g/kg
10	N-(3-phenyl-2-propenyl)-1-deoxynojirimycin	i.p.	> 0.5 g/kg
	N-(4-phenoxy-trans-2-but enyl)deoxynojirimycin	i.p.	> 0.5 g/kg
15	D-Glucaro- δ -lactam sodium salt	i.v.	> 3 g/kg
	ditto	p.o.	> 5 g/kg
	D-Glucaro- δ -lactam methyl ester	i.v.	> 5 g/kg
	ditto	p.o.	> 10 g/kg
	2,6-Di-O-(acetyl salicyloyl)-D-gluco- δ -lactam	i.p.	> 0.5 g/kg
	6-O-Triphenylmethyl-D-gluco- δ -lactam	s.c.	> 0.3 g/kg

Formulation examples of the present invention are described below.

20 (Formulation Example 1)

25	N-(3-p-Bromophenyl-2-propenyl)-1-deoxynojirimycin	50 mg
	Lactose	130 mg
	Potato starch	70 mg
	Polyvinylpyrrolidone	10 mg
	Magnesium stearate	2.5 mg

30 Lactose and potato starch are mixed and 20% ethanol solution of polyvinylpyrrolidone is added to the mixture. After uniformly wetting, the mixture is passed through a sieve of 1 mm, dried at 45 °C and again passed through a sieve of 1 mm. The thus obtained granules are kneaded with magnesium stearate and the mixture is prepared into tablets.

35 (Formulation Example 2)

40 N-(5-Phenylpentyl)-1-deoxynojirimycin is ground until it becomes particles of about 10 microns. Then, 500 mg of the particles is filled in a bial for injection. Separately, 5 mg of Tween 80 and 10 mg of purified gelatin according to the Japanese Pharmacopoeia are dissolved in 5 ml of distilled water for injection to make a dissolution liquid. Upon use, 5 ml of this dissolution solution is added to N-(5-phenylpentyl)-1-deoxynojirimycin filled in the bial. The mixture is thoroughly shaken to make a suspension and a suitable dose of the suspension is intramuscularly administered.

45 (Formulation Example 3)

50	N-(3-Phenoxypropyl)-1-deoxynojirimycin	50 mg
	Lactose	130 mg
	Potato starch	70 mg
	Polyvinylpyrrolidone	10 mg
55	Magnesium stearate	2.5 mg

55 N-(3-Phenoxypropyl)-1-deoxynojirimycin, lactose and potato starch are mixed and 20% ethanolic solution of polyvinylpyrrolidone is added to the mixture to uniformly wet the mixture. The wet mixture is

passed through a sieve of 1 mm, dried at 45 °C and again passed through a sieve of 1 mm. The thus obtained granules are kneaded with magnesium stearate to form into tablets.

5 (Formulation Example 4)

After grinding N-(3-m-methylphenoxypropyl)-1-deoxynojirimycin with a pin mill to particles of about 10 microns, 500 mg of the particles are filled up in a bial for injection. Separately, 5 mg of Tween 80 and 10 mg of gelatin purified according to the Japanese Pharmacopeia are dissolved in 5 ml of distilled water for injection to make a dissolving liquid. Upon use, 5 ml of this dissolving liquid is added to N-(3-m-methylphenoxypropyl)-1-deoxynojirimycin filled in the bial described above and the mixture is thoroughly shaken to make a suspension. A suitable dose of the suspension is intramuscularly administered.

15 (Formulation Example 5)

20	D-Glucaro- δ -lactam potassium salt	50 mg
	Lactose	130 mg
	Potato starch	70 mg
	Polyvinylpyrrolidone	10 mg
	Magnesium stearate	2.5 mg

25 D-Glucaro- δ -lactam potassium salt, lactose and potato starch are mixed and 20% ethanolic solution of polyvinylpyrrolidone is added to the mixture to uniformly wet the mixture. The mixture is passed through a sieve of 1 mm, dried at 45 °C and again passed through a sieve of 1 mm. The thus obtained granules are kneaded with magnesium stearate to form into tablets.

30 (Formulation Example 6)

35	D-Glucaro- δ -lactam ethyl ester	200 mg
	Lactose	50 mg
	CMC calcium	100 mg
	Magnesium stearate	3 mg

40 The foregoing composition was filled in hard gelatin capsules to prepare capsules.

(Formulation Example 7)

45 D-Glucaro- δ -lactam sodium salt was dissolved in distilled water for injection in a concentration of 50 mg/ml and 5 ml each per 1 ampoule was aseptically poured into each ampoule and the ampoule was sealed.

50 (Formulation Example 8)

5	6-O-[1-(p-Chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl]-D-Glucosidolactam Lactose Potato starch Polyvinylpyrrolidone Magnesium stearate	50 mg 130 mg 70 mg 10 mg 2.5 mg
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10 Lactose and potato starch were mixed and 20% ethanolic solution of polyvinylpyrrolidone was added to the mixture to wet the mixture uniformly. The mixture was passed through a sieve of 1 mm, dried at 45 °C and again passed through a sieve of 1 mm. The thus obtained granules were kneaded with magnesium stearate to form into tablets.

(Formulation Example 9)

15 After grinding 6-O-[N-(2,3-xylyl)anthranyloyl]-D-gluco- δ -lactam with a pin mill to particles of about 10 microns, 500 mg of the granules was filled in a bial for injection. Separately, 5 mg of Tween 80 and 10 mg of gelatin purified according to the Japanese Pharmacopeia were dissolved in 5 ml of distilled water for injection to make a dissolving liquid. Upon use, 5 ml of this dissolving solution was added to 6-O-[N-(2,3-xylyl)anthranyloyl]-D-gluco- δ -lactam. The mixture was thoroughly shaken to form a suspension and a suitable dose of the suspension was intramuscularly administered.

(Formulation Example 10)

25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115 120 125 130 135 140 145 150 155 160 165 170 175 180 185 190 195 200 205 210 215 220 225 230 235 240 245 250 255 260 265 270 275 280 285 290 295 300 305 310 315 320 325 330 335 340 345 350 355 360 365 370 375 380 385 390 395 400 405 410 415 420 425 430 435 440 445 450 455 460 465 470 475 480 485 490 495 500 505 510 515 520 525 530 535 540 545 550 555 560 565 570 575 580 585 590 595 600 605 610 615 620 625 630 635 640 645 650 655 660 665 670 675 680 685 690 695 700 705 710 715 720 725 730 735 740 745 750 755 760 765 770 775 780 785 790 795 800 805 810 815 820 825 830 835 840 845 850 855 860 865 870 875 880 885 890 895 900 905 910 915 920 925 930 935 940 945 950 955 960 965 970 975 980 985 990 995 1000 1005 1010 1015 1020 1025 1030 1035 1040 1045 1050 1055 1060 1065 1070 1075 1080 1085 1090 1095 1100 1105 1110 1115 1120 1125 1130 1135 1140 1145 1150 1155 1160 1165 1170 1175 1180 1185 1190 1195 1200 1205 1210 1215 1220 1225 1230 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5235 5240 5245 5250 5255 5260 5265 5270 5275 5280 5285 5290 5295 5300 5305 5310 5315 5320 5325 5330 5335 5340 5345 5350 5355 5360 5365 5370 5375 5380 5385 5390 5395 5400 5405 5410 5415 5420 5425 5430 5435 5440 5445 5450 5455 5460 5465 5470 5475 5480 5485 5490 5495 5500 5505 5510 5515 5520 5525 5530 5535 5540 5545 5550 5555 5560 5565 5570 5575 5580 5585 5590 5595 5600 5605 5610 5615 5620 5625 5630 5635 5640 5645 5650 5655 5660 5665 5670 5675 5680 5685 5690 5695 5700 5705 5710 5715 5720 5725 5730 5735 5740 5745 5750 5755 5760 5765 5770 5775 5780 5785 5790 5795 5800 5805 5810 5815 5820 5825 5830 5835 5840 5845 5850 5855 5860 5865 5870 5875 5880 5885 5890 5895 5900 5905 5910 5915 5920 5925 5930 5935 5940 5945 5950 5955 5960 5965 5970 5975 5980 5985 5990 5995 6000 6005 6010 6015 6020 6025 6030 6035 6040 6045 6050 6055 6060 6065 6070 6075 6080 6085 6090 6095 6100 6105 6110 6115 6120 6125 6130 6135 6140 6145 6150 6155 6160 6165 6170 6175 6180 6185 6190 6195 6200 6205 6210 6215 6220 6225 6230 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7235 7240 7245 7250 7255 7260 7265 7270 7275 7280 7285 7290 7295 7300 7305 7310 7315 7320 7325 7330 7335 7340 7345 7350 7355 7360 7365 7370 7375 7380 7385 7390 7395 7400 7405 7410 7415 7420 7425 7430 7435 7440 7445 7450 7455 7460 7465 7470 7475 7480 7485 7490 7495 7500 7505 7510 7515 7520 7525 7530 7535 7540 7545 7550 7555 7560 7565 7570 7575 7580 7585 7590 7595 7600 7605 7610 7615 7620 7625 7630 7635 7640 7645 7650 7655 7660 7665 7670 7675 7680 7685 7690 7695 7700 7705 7710 7715 7720 7725 7730 7735 7740 7745 7750 7755 7760 7765 7770 7775 7780 7785 7790 7795 7800 7805 7810 7815 7820 7825 7830 7835 7840 7845 7850 7855 7860 7865 7870 7875 7880 7885 7890 7895 7900 7905 7910 7915 7920 7925 7930 7935 7940 7945 7950 7955 7960 7965 7970 7975 7980 7985 7990 7995 8000 8005 8010 8015 8020 8025 8030 8035 8040 8045 8050 8055 8060 8065 8070 8075 8080 8085 8090 8095 8100 8105 8110 8115 8120 8125 8130 8135 8140 8145 8150 8155 8160 8165 8170 8175 8180 8185 8190 8195 8200 8205 8210 8215 8220 8225 8230 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10190 10195 10200 10205 10210 10215 10220 10225 10230 10235 10240 10245 10250 10255 10260 10265 10270 10275 10280 10285 10290 10295 10300 10305 10310 10315 10320 10325 10330 10335 10340 10345 10350 10355 10360 10365 10370 10375 10380 10385 10390 10395 10400 10405 10410 10415 10420 10425 10430 10435 10440 10445 10450 10455 10460 10465 10470 10475 10480 10485 10490 10495 10500 10505 10510 10515 10520 10525 10530 10535 10540 10545 10550 10555 10560 10565 10570 10575 10580 10585 10590 10595 10600 10605 10610 10615 10620 10625 10630 10635 10640 10645 10650 10655 10660 10665 10670 10675

insoluble matters, the filtrate was concentrated to give 5 g of the residue. The residue was dissolved in ethyl acetate and water. The ethyl acetate phase was separated and concentrated to give 3.8 g of oil. The oil was separated by silica gel chromatography (chloroform : methanol = 10 : 1) to give 1.6 g of the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.46).

5

(Preparation Example 2)

10 2-O-(6-Methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam and 2,6-di-O-(6-Methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam:

15 6-Methoxy- α -methyl-2-naphthaleneacetic acid, 11.5 g was dissolved in 160 ml of methylene chloride and 0.1 ml of dimethylformamide and 7.3 ml of thionyl chloride was added to the solution. The mixture was stirred at room temperature for 5 hours. After the solvent was distilled off, toluene was added thereto and the mixture was further concentrated to dryness to give the acid chloride.

20 D-Gluco- δ -lactam, 5.85 g, was dissolved in 100 ml of dimethylformamide and 12 ml of pyridine was added to the solution. The mixture was cooled to 5°C and the acid chloride of 6-methoxy- α -methyl-2-naphthaleneacetic acid was added to the mixture. The resulting mixture was stirred at room temperature overnight. After filtering off insoluble matters, the solvent was distilled off and toluene was further added to the residue. The mixture was further concentrated to dryness to give the residue. Ethyl acetate and water were added to the residue. Firstly insoluble matters were taken by filtration and 1.8 g of 2-O-(6-methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), R_f = 0.26) was obtained.

25 Then, the ethyl acetate phase was separated and concentrated to make the amount of ethyl acetate 150 ml. Insoluble matters were taken by filtration and 5.86 g of 2,6-di-O-(6-methoxy- α -methyl-2-naphthaleneacetyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), R_f = 0.48) was obtained.

30 (Preparation Example 3)

35 6-O-(1-(p-Chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetyl)-D-gluco- δ -lactam and 2,6-di-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetyl)-D-gluco- δ -lactam:

40 Indomethacin, 5.37 g, was dissolved in 50 ml of 1,2-dichloroethane and 0.1 ml of dimethylformamide and 2.25 ml of thionyl chloride was added to the solution. The mixture was stirred at 60°C for 1.5 hours. After concentration, toluene was added and the mixture was further concentrated to give the acid chloride of Indomethacin.

45 D-Gluco- δ -lactam, 2 g, was dissolved in a mixture of 50 ml of dimethylformamide and 1.7 ml of pyridine and the solution was cooled to 5°C. Then, a solution of the acid chloride of Indomethacin in 20 ml of dimethylformamide was added to the solution and the mixture was stirred at room temperature overnight. The solvent was distilled off and separation was made directly by means of silica gel column chromatography (chloroform : methanol = 20 : 1) to give 0.7 g of 6-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetyl)-D-gluco- δ -lactam and 0.7 g of 2-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), R_f = 0.33) as well as 2.16 g of 2,6-di-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methyl-3-indoleacetyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), R_f = 0.56).

50

(Preparation Example 4)

55 2,6-Di-O-(3-benzoylhydratropoyl)-D-gluco- δ -lactam:

Ketoprofen, 5.08 g, was dissolved in a mixture of 50 ml of 1,2-dichloroethane and 0.1 ml of dimethylformamide and 2.95 ml of thionyl chloride was added to the solution. The mixture was stirred at 60°C for 2 hours. The solvent was distilled off and toluene was added to the residue. Toluene was further

distilled off to give the acid chloride of Ketoprofen.

D-Glucos- δ -lactam, 3.54 g, was dissolved in 35 ml of dimethylformamide and 4 ml of pyridine. The solution was cooled to 5°C. A solution of the acid chloride of Ketoprofen in 20 ml of dimethylformamide was added to the solution. The solvent was distilled off and 40 ml of water, 40 ml of methanol and 150 ml of ethyl ether were added to the residue to make a dual layer solution. Thereafter the ethereal phase was separated. After the ethereal phase was further washed with a sodium bicarbonate aqueous solution, The solvent was distilled off and then separation was performed by means of silica gel column chromatography (chloroform : methanol = 40 : 1) to give 1.98 g of the title compound (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), Rf = 0.44).

10

(Preparation Example 5)

15 6-O-(N-(2,3-Xylyl)anthranyloyl)-D-gluco- δ -lactam:

D-Glucos- δ -lactam, 5.31 g, and 7.23 g of mefenamic acid were dissolved in 60 ml of dimethylformamide and, 6.3 ml of triethylamine and then 6.6 ml of diethyl cyanophosphonate were added to the solution. The mixture was stirred at room temperature overnight. After distilling the solvent off, ethyl ether was added to the residue and the mixture was stirred at room temperature. Thereafter insoluble matters were taken by filtration. A small quantity of methanol was added to the filtered solid to make a solution. Then, ethyl ether and isopropyl ether were added to the solution. The precipitates were taken by filtration. The obtained precipitates were further dissolved in methanol to make a solution. Isopropyl ether was added to the solution to again make a methanolic solution. Isopropyl ether was added to again cause precipitation and give 1.42 g of the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), Rf = 0.51).

30

(Preparation Example 6)

35 6-O-(Acetylsalicyloyl)-D-gluco- δ -lactam, 2,6-di-O-(acetylsalicyloyl)-D-gluco- δ -lactam and 2,3,6-tri-O-(acetylsalicyloyl)-D-gluco- δ -lactam:

D-Glucos- δ -lactam, 3.52 g, was suspended in a mixture of 50 ml of pyridine and 15 ml of N,N-dimethylformamide. Under ice cooling, a solution of 8.0 g of O-acetylsalicyloyl chloride in 50 ml of N,N-dimethylformamide was dropwise added to the suspension. After stirring at 0 to 5°C for an hour, the temperature was reverted to room temperature. After reacting overnight while stirring, insoluble matters were filtered off and the filtrate was concentrated to dryness. The residue was purified by silica gel column chromatography (chloroform : methanol = 10 : 1) to give 0.3 g of 6-O-(acetylsalicyloyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), Rf = 0.11), 3.0 g of 2,6-di-O-(acetylsalicyloyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), Rf = 0.41) and 0.4 g of 2,3,6 tri-O-(acetylsalicyloyl)-D-gluco- δ -lactam (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), Rf = 0.53), respectively.

45

(Preparation Example 7)

50 6-O-(3,5-Difluoromethylbenzhydryl)-D-gluco- δ -lactam:

The hydrazone compound, 1.0 g, synthesized from 3,5-difluoro-methylbenzophenone in a conventional manner was dissolved in 20 ml of ethyl acetate and 3.0 g of nickel peroxide was added to the solution. The mixture was stirred at room temperature for an hour.

55 After removing insoluble matters, ethyl acetate was distilled off below 30°C to give 0.95 g of the diazo compound.

The obtained diazo compound, 0.95 g, and 500 mg of 2,3,4-tri-O-acetyl-D-gluco- δ -lactam were dissolved in 10 ml of 1,2-dichloroethane. The solution was cooled to 5°C. While stirring, a solution of 40 mg of

trifluoroboric acid etherate in 1 ml of 1,2-dichloroethane was dropwise added to the solution slowly in argon flow. After completion of the dropwise addition, the mixture was stirred at 5°C for 30 minutes and then concentrated. The thus obtained liquid was purified by silica gel column chromatography (toluene : ethyl acetate = 2 : 1) to give 540 mg of 2,3,4-tri-O-acetyl-6-O-(3,5-ditrifluoromethylbenzhydryl)-D-gluco- δ -lactam-⁵ (silica gel thin layer chromatography (toluene : ethyl acetate = 1 : 1), R_f = 0.34).

The thus obtained 2,3,4-tri-O-acetyl 6-O-substituted compound, 270 mg, was dissolved in 4 ml of methanol and a methanolic solution of sodium methoxide freshly prepared was added to the solution at any time followed by stirring at room temperature.

The reaction was monitored by silica gel thin layer chromatography (chloroform : methanol = 4 : 1).

After completion of the reaction, the same amount of water as that of the reaction solution was added to the reaction solution. Thereafter, the mixture was treated with Amberlist 15 to remove sodium ions and then concentrated to dryness. To the residue was added 5 ml of diethyl ether. The mixture was stirred at room temperature for 30 minutes. Insoluble solid was taken by filtration to give 150 mg of the title compound-¹⁰ (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.61; mass spectrum (F.D. mass) m/z: 479 (M⁺)).

(Preparation Example 8)

²⁰ 6-O-(3,3'-Ditrifluoromethylbenzhydryl)-D-gluco- δ -lactam:

Using the hydrazone compound for 3,3'-ditrifluoromethyl-benzhydrylation instead of the hydrazone compound for 3,5-ditrifluoromethylbenzhydrylation in Preparation Example 7, the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.51; mass spectrum (F.D. mass) m/z : 480 (M⁺)) was obtained in a manner similar to Preparation Example 7.

(Preparation Example 9)

³⁰ 6-O-p-Phenylbenzhydryl-D-gluco- δ -lactam:

Using the hydrazone compound for p-phenylbenzhydrylation instead of the hydrazone compound for 3,5-ditrifluoromethylbenzhydrylation in Preparation Example 7, the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.64; mass spectrum (F.D. mass) m/z : 419 (M⁺)) was obtained in a manner similar to Preparation Example 7.

⁴⁰ (Preparation Example 10)

6-O-(1- β -Naphthylethyl)-D-gluco- δ -lactam:

⁴⁵ Using the hydrazone compound for 1- β -naphthylethylation instead of the hydrazone compound for 3,5-ditrifluoromethylbenzhydrylation in Preparation Example 7, the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.4; mass spectrum (F.D. mass) m/z : 331 (M⁺)) was obtained in a manner similar to Preparation Example 7.

⁵⁰ (Preparation Example 11)

6-O-(1-(3,4-Dimethoxyphenyl)ethyl)-D-gluco- δ -lactam:

⁵⁵ Using the hydrazone compound for (3,4-dimethoxyphenyl)ethylation instead of the hydrazone compound for 3,5-ditrifluoromethylbenzhydrylation in Preparation Example 7, the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.43; mass spectrum (F.D. mass) m/z : 341

(M⁺) was obtained in a manner similar to Preparation Example 7.

(Preparation Example 12)

5

6-O-(3,4-Dichlorobenzhydryl)-D-gluco- δ -lactam:

Using the hydrazone compound for 3,4-dichlorobenzhydrylation instead of the hydrazone compound for 10 3,5-ditrifluoromethylbenzhydrylation in Preparation Example 7, the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 0.46; mass spectrum (F.D. mass) m/z : 411 (M⁺)) was obtained in a manner similar to Preparation Example 7.

15 (Preparation Example 13)

3,6-Di-O-trityl-D-gluco- δ -lactam:

20 D-Gluco- δ -lactam, 1.7 g, and 5.6 g of trityl chloride were mixed with 50 ml of pyridine. The mixture was stirred overnight with heating at 50 °C. After pyridine was distilled off, chloroform and saturated aqueous sodium bicarbonate solution were added to the residue followed by extraction with chloroform 3 times. After drying the chloroform phase over anhydrous potassium carbonate, chloroform was distilled off and the residue was purified by silica gel thin layer chromatography (chloroform : methanol = 70 : 1) to give 0.8 g 25 of the title compound (silica gel thin layer chromatography (chloroform : methanol = 50 : 1), R_f = 0.25; mass spectrum (F.D. mass) m/z : 660 (M⁺)).

(Preparation Example 14)

30

6-O-(3,3'-Ditrifluoromethyltrityl)-D-gluco- δ -lactam:

35 D-Gluco- δ -lactam, 1.06 g, and 3.0 g of 3,3'-ditrifluoromethyltrityl chloride were dissolved in 40 ml of pyridine. The mixture was heated at 60 °C overnight while stirring. After pyridine was distilled off, ethyl acetate and saturated sodium bicarbonate aqueous solution were added to the residue. After the aqueous phase was further saturated with sodium chloride, the system was extracted with ethyl acetate 3 times. After drying the ethyl acetate phase over anhydrous potassium carbonate, the solvent was distilled off and the residue was purified by silica gel column chromatography (chloroform : methanol = 20 : 1) to give 600 mg 40 of the title compound (silica gel thin layer chromatography (chloroform : methanol = 10 : 1), R_f = 0.23).

(Preparation Example 15)

45

6-O-(3,5-Ditrifluoromethyltrityl)-D-gluco- δ -lactam:

45 Using 3,5-ditrifluoromethyltrityl chloride instead of 3,3'-ditrifluoromethyltrityl chloride in Preparation Example 14, the title compound (silica gel thin layer chromatography (chloroform : methanol = 4 : 1), R_f = 50 0.53, mass spectrum (F.D. mass) m/z : 555 (M⁺)) was obtained in a manner similar to Preparation Example 14.

(Preparation Example 16)

55

6-O-(4-tert-butyltrityl)-D-gluco- δ -lactam:

Using 4-tert-butyltrityl chloride instead of 3,3'-difluoromethyltrityl chloride in Preparation Example 14, the title compound (silica gel thin layer chromatography (chloroform-methanol = 6 : 1), R_f = 0.38; mass spectrum (F.D. mass) m/z : 475 (M^+)) was obtained in a manner similar to Preparation Example 14.

The effectiveness of the compounds according to the present invention was determined by the following 5 method.

Test Methods:

10 Based on the method described previously (Method in Cancer Research, 15, 399-439, 1978), a subline of high metastatic potential (high pulmonary colonization) was selected from murine melanoma B16 by repeating the i.v. transplantation of the tumor cells in BDF₁ mice and the isolation of pulmonary metastatic clones.

15 Evaluation of the metastasis inhibiting activity was performed based on the method described previously (Proc. Natl Acad. Sci. USA, 83, 1752-1756, 1986, Cancer Research, 46, 858-862, 1986).

Firstly, the B16 subline of high metastatic potential was inoculated into Dulbecco's ME medium (DME medium) supplemented with 10 % bovine fetal serum and the compound represented by general formula (I) described above was added thereto. Incubation was performed at 37 °C for 2 to 4 days in the presence of 5% CO₂ and proliferated cells were detached from the Petri dish with trypsin-EDTA solution. The cells were 20 suspended in divalent cation-free Dulbecco's phosphate-buffered saline (PBS⁻) in 1 × 10⁶ cells/ml as viable cells.

This suspension, 0.1 ml, was intravenously injected in the mouse tail vein. After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of metastatic nodules of the B16 subline of high pulmonary metastatic potential on the surface of and in the lungs was counted.

25

(Test Example 1)

30 Cytotoxic activity of N-(3-phenylpropyl)-1-deoxynojirimycin:

The B16 subline of high pulmonary metastatic potential and murine tumor cell line L 929 were cultured in DME medium supplemented with 10% bovine fetal serum at 37 °C in the presence of 5% CO₂. Then, cells were detached from the Petri dish with trypsin-EDTA solution. The B16 subline and L 929 were 35 suspended in the medium described above in 1 × 10⁴ cells/ml and 1 × 10³ cells/ml, respectively. Each suspension, 150 µl, was added to 50 µl of N-(3-phenylpropyl)-1-deoxynojirimycin solution or Adriamycin (control) solution. Incubation was performed for 3 days for the B16 subline and for 4 days for L 929 strain. Cell growth was observed with the aid of an inverted microscope to determine the cytotoxicity. The results are shown in Table 2.

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Table 2

Cell Line: B16 Subline of High Pulmonary Metastatic Potential					
Drug Added	Concentration	Growth			
None		+			
N-(3-Phenylpropyl)-1-deoxynojirimycin	30 μ g/ml 100 μ g/ml 300 μ g/ml	+	+	\pm	
Adriamycin (control)	0.1 μ g/ml	-			
Cell Line: L 929					
Drug Added	Concentration	Growth			
None		+			
N-(3-Phenylpropyl)-1-deoxynojirimycin	0.5 μ g/ml 5 μ g/ml 50 μ g/ml 100 μ g/ml	+	+	+	+
Adriamycin (control)	1 μ g/ml	-			
In the table:					
+ indicates growth;					
\pm indicates growth in which marked inhibition is noted.					
- indicates death.					

As shown in Table 2, N-(3-phenylpropyl)-1-deoxynojirimycin which is the effective ingredient of the present invention did not show marked cytotoxicity against the B16 subline of high pulmonary metastatic potential and L 929 even at a concentration as high as 100 μ g/ml.

35 (Test Example 2)

Inhibition of metastasis by N-(3-phenylpropyl)-1-deoxynojirimycin:

40 The B16 subline of high pulmonary metastatic potential was inoculated into DME medium supplemented with 10% bovine fetal serum and 30, 100 or 300 μ g/ml of N-(3-phenylpropyl)-1-deoxynojirimycin was added thereto followed by incubation at 37°C for 3 days in the presence of 5% CO₂. The cells were detached from the Petri dish with trypsin-EDTA solution. The cells were suspended in PBS- in 1 \times 10⁶ cells/ml as viable cells. This suspension, 0.1 ml, was intravenously injected in the tail vein of the mouse 45 BDF₁ (8 week age, male). After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of colonies of the B16 subline formed on the surface of and in the lungs was counted. The results are shown in Table 3.

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Table 3

Drug Added	Concentration	Pulmonary Metastasis (no.)				
		Mouse 1	Mouse 2	Mouse 3	Mean \pm Standard Deviation	%
None		29	32	35	32 \pm 3.0	100
N-(3-Phenylpropyl)-1-deoxynojirimycin:	30 μ g/ml	9	22	37	22.7 \pm 14.0	68.8
	100 μ g/ml	4	4	6	4.7 \pm 1.2	14.7
	300 μ g/ml	0	0	3	1 \pm 1.7	3.1

As shown in Table 3, N-(3-phenylpropyl)-1-deoxynojirimycin that is the effective ingredient of the present invention shows decrease in the number of colonies formed on the surface of and in the lungs.

(Test Example 3)

Inhibition of metastasis by N-(3-phenyl-2-propenyl)-1-deoxy-nojirimycin and N-(3-p-chlorophenyl-3-methyl-2-propenyl)-1-deoxynojirimycin:

Inhibition of metastasis was examined in a manner similar to Test Example 2 except that N-(3-phenyl-2-propenyl)-1-deoxy-nojirimycin and N-(3-p-chlorophenyl-3-methyl-2-propenyl)-1-deoxynojirimycin were used instead of N-(3-phenylpropyl)-1-deoxynojirimycin in Test Example 2. In this test example, however, 2.5×10^5 cells each of the B16 subline were transplanted to 3 mice for each group. The results are shown in Table 4.

Table 4

Drug Added	Concentration	Pulmonary Metastasis (no.) (Mean \pm Standard derivation)
None		109 \pm 32.0
N-(3-Phenyl-2-propenyl)-1-deoxynojirimycin:	30 μ g/ml	16.8 \pm 12.6
N-(3-p-Chlorophenyl-3-methyl-2-propenyl)-1-deoxynojirimycin:	30 μ g/ml	14.0 \pm 8.9

As shown in Table 4, metastasis of the B16 subline of high pulmonary metastatic potential to the lungs was markedly inhibited by N-(3-phenyl-2-propenyl)-1-deoxynojirimycin or N-(3-p-chlorophenyl-3-methyl-2-propenyl)-1-deoxynojirimycin.

(Test Example 4)

Cytotoxic activity of N-(4-phenoxy-trans-2-but enyl)-1-deoxynojirimycin:

The B16 subline of high pulmonary metastatic potential and murine tumor cell line L 929 were cultured in DME medium supplemented with 10% bovine fetal serum at 37°C in the presence of 5% CO₂. Then, cells were detached from the Petri dish with trypsin-EDTA solution. The B16 subline and L 929 were suspended in the medium described above in 1×10^4 cells/ml and 1×10^3 cells/ml, respectively. Each suspension, 150 μ l, was added to 50 μ l of N-(4-phenoxy-trans-2-but enyl)-1-deoxynojirimycin solution or Adriamycin (control) solution. Incubation was performed for 3 days in the B16 subline and for 4 days in L 929. Cell growth was observed with the aid of an inverted microscope to determine the cytotoxicity. The results are shown in Table 5.

Table 5

Cell Line: B16 Subline of High Pulmonary Metastatic Potential		
Drug Added	Concentration	Growth
None		+
N-(4-Phenoxy-trans-2-but enyl)-1-deoxynojirimycin	3 μ g/ml 10 μ g/ml 30 μ g/ml	+
Adriamycin (control)	0.1 μ g/ml	-
Cell Line: L 929		
Drug Added	Concentration	Growth
None		+
N-(4-Phenoxy-trans-2-but enyl)-1-deoxynojirimycin	3 μ g/ml 30 μ g/ml 100 μ g/ml	+
Adriamycin (control)	1 μ g/ml	-
In the table:		
+ indicates growth;		
- indicates death.		

As shown in Table 5, N-(4-phenoxy-trans-2-but enyl)-1-deoxynojirimycin which is the effective ingredient of the present invention did not show the marked cytotoxicity against the B16 subline of high pulmonary metastatic potential and L 929 even at a concentration as high as 30 μ g/ml.

(Test Example 5)

Inhibition of metastasis by N-(4-phenoxytrans-2-but enyl)-1-deoxynojirimycin:

The B16 subline of high pulmonary metastatic potential was inoculated into DME medium supplemented with 10% bovine fetal serum and 30 μ g/ml of N-(4-phenoxy-trans-2-but enyl)-1-deoxynojirimycin was added thereto followed by incubation at 37 °C for 3 days in the presence of 5% CO₂. The cells were detached from the Petri dish with trypsin-EDTA solution. The cells were suspended in PBS- in 1 \times 10⁶ cells/ml as viable cells. This suspension, 0.1 ml, was intravenously injected in the tail vein of the mouse BDF₁ (8 week age, male). After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of colonies of the B16 subline formed on the surface of and in the lung was counted. The results are shown in Table 6.

Table 6

Drug Added	Concentration	Pulmonary Metastasis (no.)					
		Mouse 1	Mouse 2	Mouse 3	Mean \pm Standard Deviation	% Derivation	
None	10 μ g/ml	120	141	145	135 \pm 13.4	100	
N-(4-Phenoxy-trans-2-butene)-1-deoxy- <i>nor</i> lirimycin:		14	48	52	38 \pm 20.9	28.1	

As shown in Table 6, in the test group of N-(4-phenoxy-trans-2-butenyl)-1-deoxynojirimycin that was the effective ingredient of the present invention, the number of colonies on the surface of and in the lungs was decreased.

5

(Test Example 6)

Inhibition of metastasis by N-(2-phenoxyethyl)-1-deoxynojirimycin:

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Inhibition of metastasis was examined in a manner similar to Test Example 5 except that N-(2-phenoxyethyl)-1-deoxynojirimycin was used instead of N-(4-phenoxy-trans-2-butenyl)-1-deoxynojirimycin in Test Example 5. The results are shown in Table 7.

15

Table 7

20

Drug Added	Concentration	Pulmonary Metastasis (no.) (Mean±Standard Deviation)
None N-(2-Phenoxyethyl)-1-deoxynojirimycin:	100 µg/ml	86 ± 22.1 7 ± 2.1

25

As shown in Table 7, metastasis of the B16 subline of high pulmonary metastatic potential to the lungs was markedly inhibited by N-(2-phenoxyethyl)-1-deoxynojirimycin which was the effective ingredient of the present invention.

(Test Example 7)

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Cytotoxic activity of D-glucaro- δ -lactam calcium salt:

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The B16 subline of high pulmonary metastatic potential and murine tumor cell line L 929 were cultured in DME medium supplemented with 10% bovine fetal serum at 37°C in the presence of 5% CO₂. Then, cells were detached from the Petri dish with trypsin-EDTA solution. The B16 subline and L 929 were suspended in the medium described above in 1 × 10⁴ cells/ml and 1 × 10³ cells/ml, respectively. Each suspension, 150 µl, was added to 50 µl of D-glucaro- δ -lactam calcium salt solution or Adriamycin (control) solutions. Incubation was performed for 3 days in the B16 subline and for 4 days in L 929. Cell growth was observed with the aid of an inverted microscope to determine the cytotoxicity.

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The results are shown in Table 8.

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Table 8

Cell Line: B16 Subline of High Pulmonary Metastatic Potential		
Drug Added	Concentration	Growth
None		+
D-Glucaro- δ -lactam calcium salt	10 μ g/ml 30 μ g/ml	+
Adriamycin (control)	0.1 μ g/ml	-
Cell Line: L 929		
Drug Added	Concentration	Growth
None		+
D-Glucaro- δ -lactam calcium salt	10 μ g/ml 30 μ g/ml	+
Adriamycin (control)	1 μ g/ml	-
In the table:		
+ indicates growth;		
- indicates death.		

As shown in Table 8, D-glucaro- δ -lactam calcium salt which was the effective ingredient of the present invention did not show marked cytotoxicity against the B16 subline and L 929 even at a concentration as high as 30 μ g/ml.

(Test Example 8)

Inhibition of metastasis by of D-glucaro- δ -lactam calcium salt:

The B16 subline of high pulmonary metastatic potential was inoculated into DME medium supplemented with 10% bovine fetal serum and 10 μ g/ml of D-glucaro- δ -lactam calcium salt was added thereto followed by incubation at 37°C for 3 days in the presence of 5% CO₂. The cells were detached from the Petri dish with trypsin-EDTA solution. The cells were suspended in PBS- in 1 \times 10⁶ cells/ml as viable cells. This suspension, 0.1 ml, was intravenously injected in the tail vein of the mouse BDF₁ (8 week age, male). After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of the colonies formed on the surface of and in the lung was counted. The results are shown in Table 9.

Table 9

Drug Added	Concentration	Pulmonary Metastasis (no.)				
		Mouse 1	Mouse 2	Mouse 3	Mean \pm Standard Deviation	%
None		70	69	68	69 \pm 1.0	100
D-Glucaro- δ -lactam calcium salt	10 μ g/ml	7	4	0	3.7 \pm 3.5	5.4

As shown in Table 9, metastasis of the B16 subline of high pulmonary metastatic potential was markedly inhibited by the treatment with D-glucaro- δ -lactam calcium salt that is the effective ingredient of the present invention.

(Test Example 9)

Inhibition of metastasis by D-glucaro- δ -lactam methyl ester and D-glucaro- δ -lactam ethyl ester:

5

Inhibition of metastasis was examined in a manner similar to Test Example 8 except that D-glucaro- δ -lactam methyl ester and D-glucaro- δ -lactam ethyl ester were used instead of D-glucaro- δ -lactam calcium salt in Test Example 8. The results are shown in Table 10.

10

Table 10

15

Drug Added	Concentration	Pulmonary Metastasis(no.) (Mean \pm Standard Deviation)
None		82 \pm 11.2
D-Glucaro- δ -lactam methyl ester:	30 μ g/ml	21 \pm 8.5
D-Glucaro- δ -lactam ethyl ester:	30 μ g/ml	13 \pm 5.4

20

As shown in Table 10, metastasis of the B16 subline to the lungs was markedly inhibited by D-glucaro- δ -lactam methyl ester or D-glucaro- δ -lactam ethyl ester which was the effective ingredients of the present invention.

25

(Test Example 10)

Cytotoxic activity of 2,6 di-O-[1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl]-D-gluco- δ -lactam:

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The B16 subline of high pulmonary metastatic potential and murine tumor cell line L 929 were cultured in DME medium supplemented with 10% bovine fetal serum at 37°C in the presence of 5% CO₂. Then, cells were detached from the Petri dish with trypsin-EDTA solution. The B16 subline and L 929 were suspended in the medium described above in 1 x 10⁴ cells/ml and 1 x 10³ cells/ml, respectively. Each suspension, 150 μ l, was added to 50 μ l of 2,6-di-O-(1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl)-D-gluco- δ -lactam solution or and Adriamycin (control) solutions. Incubation was performed for 3 days in the B16 subline and for 4 days in L 929. Cell growth was observed with the aid of an inverted microscope to determine the cytotoxicity. The results are shown in Table 11.

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Table 11

Cell Line: B16 Subline of High Pulmonary Metastatic Potential			
Drug Added	Concentration	Growth	
None		+	
2,6-Di-O-[1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl]-D-Gluco- δ -lactam	10 μ g/ml	+	
Adriamycin (control)	0.1 μ g/ml	-	
Cell Line: L 929			
Drug Added	Concentration	Growth	
None		+	
2,6-Di-O-[1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl]-D-Gluco- δ -lactam	3 μ g/ml	+	
	10 μ g/ml	+	
	30 μ g/ml	+	
Adriamycin (control)	1 μ g/ml	-	
In the table:			
+ indicates growth;			
- indicates death.			

As shown in Table 11, 2,6-di-O-[1-(p-chlorobenzoyl)-5-methoxy-2-methylindole-3-acetyl]-D-Gluco- δ -lactam which was the effective ingredient of the present invention did not show marked cytotoxicity against the B16 subline and L 929 even at a concentration as high as 10 μ g/ml.

(Test Example 11)

Inhibition of metastasis by 2,6-di-O-(acetylsalicyloyl)-D-gluco- δ -lactam:

The B16 subline of high pulmonary metastatic potential was inoculated into DME medium supplemented with 10% bovine fetal serum and 10 μ g/ml of 2,6-di-O-(acetylsalicyloyl)-D-gluco- δ -lactam was added thereto followed by incubation at 37 °C for 3 days in the presence of 5% CO₂. The cells were detached from the Petri dish with trypsin-EDTA solution. The cells were suspended in PBS- in 1 x 10⁶ cells/ml as viable cells. This suspension, 0.1 ml, was intravenously injected in the tail vein of the mouse, BDF₁ (8 week age, male; 1 group, 3 mice). After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of the colonies of the B16 subline formed on the surface of and in the lungs was counted. The results are shown in Table 12.

Table 12

Drug Added	Concentration	Pulmonary Metastasis (no.) (Mean \pm Standard Deviation)
None		53 \pm 22.4
2,6-Di-O-(acetylsalicyloyl)-D-gluco- δ -lactam:	10 μ g/ml	11.2 \pm 3.5

As shown in Table 12, metastasis of the B16 subline of high pulmonary metastasis potential was markedly inhibited by 2,6-di-O-(acetylsalicyloyl)-D-gluco- δ -lactam which was the effective ingredients of the present invention.

55

(Test Example 12)

Cytotoxic activity of 6-O-triphenylmethyl-D-gluco- δ -lactam:

The B16 subline of high pulmonary metastatic potential and murine tumor cell line L 929 were cultured in DME medium supplemented with 10% bovine fetal serum at 37 °C in the presence of 5% CO₂. Then, 5 cells were detached from the Petri dish with trypsin-EDTA solution. The B16 subline and L 929 were suspended in the medium described above in 1 x 10⁴ cells/ml and 1 x 10³ cells/ml, respectively. Each suspension, 150 μ l, was added to 50 μ l of 6-O-triphenylmethyl-D-gluco- δ -lactam solution or Adriamycin (control) solutions. Incubation was performed for 3 days in the B16 subline and for 4 days in L 929. Cell growth was observed with the aid of an inverted microscope to determine the cytotoxicity. The results are 10 shown in Table 13.

Table 13

Cell Line: B16 Subline of High Pulmonary Metastatic Potential		
Drug Added	Concentration	Growth
None		+
6-O-Triphenylmethyl-D-Gluco- δ -lactam	3 μ g/ml 10 μ g/ml 30 μ g/ml	+
Adriamycin (control)	0.1 μ g/ml	-
Cell Line: L 929		
Drug Added	Concentration	Growth
None		+
6-O-Triphenylmethyl-D-Gluco- δ -lactam	10 μ g/ml 30 μ g/ml	+
Adriamycin (control)	1 μ g/ml	-
In the table:		
+ indicates growth;		
- indicates death.		

As shown in Table 13, 6-O-triphenylmethyl-D-gluco- δ -lactam which was the effective ingredient of the present invention did not show marked cytotoxicity against the B16 subline and L 929 even at a 40 concentration as high as 30 μ g/ml.

(Test Example 13)

45 Inhibition of metastasis by 6-O-triphenylmethyl-D-gluco- δ -lactam:

The B16 subline of high pulmonary metastatic potential was inoculated into DME medium supplemented with 10% bovine fetal serum and 30 μ g/ml of 6-O-triphenylmethyl-D-gluco- δ -lactam was added thereto 50 followed by incubation at 37 °C for 3 days in the presence of 5% CO₂. The cells were detached from the Petri dish with trypsin-EDTA solution. The cells were suspended in PBS⁻ in 1 x 10⁶ cells/ml as viable cells. This suspension, 0.1 ml, was intravenously injected in the tail vein of the mouse BDF₁ (8 week age, male; 1 group, 3 mice). After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of colonies of the B16 subline on the surface of and in the lungs was counted. The results are shown in Table 55 14.

Table 14

Drug Added	Concentration	Pulmonary Metastasis (no.) (Mean \pm Standard Deviation)
None		92 \pm 21.3
6-O-Triphenylmethyl-D-gluco- δ -lactam	30 μ g/ml	17 \pm 8.5

5 As shown in Table 14, metastasis of the B16 subline to the lungs was markedly inhibited by 6-O-
10 triphenylmethyl D-gluco- δ -lactam which was the effective ingredient of the present invention.

(Test Example 14)

15 Inhibition of metastasis by 1-deoxynojirimycin:

20 The B16 subline of high pulmonary metastatic potential was inoculated into DME medium supplemented with 10% bovine fetal serum and 30 μ g/ml of 1-deoxynojirimycin was added thereto followed by incubation at 37°C for 3 days in the presence of 5% CO₂. In this case, cytotoxicity by this substance was hardly observed. Next, the cells were detached from the Petri dish with trypsin-EDTA solution. The cells were suspended in PBS- in 1 \times 10⁶ cells/ml as viable cells. This suspension, 0.1 ml, was intravenously injected in the tail vein of the mouse BDF₁ (8 week age, male; 1 group, 3 mice). After feeding for 14 days, the mouse was sacrificed to excise the lungs. The number of colonies of the B16 subline on the surface of
25 and in the lungs was counted. The results are shown in Table 15.

Table 15

Drug Added	Pulmonary Metastasis (no.) (Mean \pm Standard Deviation)
None	119 \pm 37
1-Deoxynojirimycin (30 μ g/ml)	31 \pm 11

35 As shown in Table 15, the number of colonies on the surface of and in the lungs was markedly inhibited by treatment with 1-deoxynojirimycin which was the effective ingredient of the present invention.

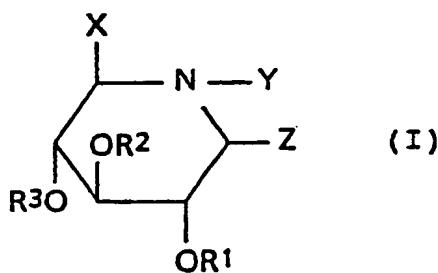
While the invention has been described in detail and with reference to specific embodiments thereof, it will be apparent to one skilled in the art that various changes and modifications can be made therein without
40 departing from the spirit and scope thereof.

In the specification and in the claims "alkyl" preferably is a lower alkyl having 1 to 5 carbon atoms, "lower alkyl" stands for alkyl groups having preferably 1 to 5 carbon atoms, "aryl" preferably is phenyl or naphthyl, "aralkyl" preferably is aryl as defined above having an alkyl group containing preferably 1 to 5 carbon atoms, "acyl" preferably is an acyl group having totally 1 to 6 carbon atoms, like formyl, acetyl, propionyl or butyryl, and the substituents on the alkyl, aryl, and aralkyl groups preferably are one or more substituents, selected from halogen atoms, alkyl groups, preferably having 1 to 5 carbon atoms, amino groups, cyano groups, nitro groups, nitrile groups etc., "lower alkoxy" preferably is a group containing an alkyl group having 1 to 5 carbon atoms, the alkyl group in the "carboxy alkyl" radicals preferably also contains 1 to 5 carbon atoms and the same applies to the alkyl groups in the "carboalkoxy alkyl" radicals.

50

Claims

1. A cancer cell metastasis inhibitor comprising as an effective ingredient a compound represented by
55 formula (I):



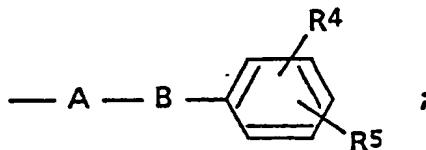
10

In formula (I):

a)

75 X : -CH₂OH;
 Y : hydrogen atom or

20



25 A : hydrocarbon group having 3 to 5 carbon atoms which may or may not contain a double bond;
 B : bond or, oxygen atom or sulfur atom;

R⁴, R⁵ : independently hydrogen atom, halogen atom or
 a lower alkyl group;

Z : hydrogen atom;

30 R¹, R², R³ : hydrogen atom

b)

X : -COOR⁶ (R⁶ : hydrogen atom or an alkyl group having 1 to 8 carbon atoms, pivaloyloxymethyl group or (5-methyl-2-oxo-1,3-dioxole-4-yl)methyl group);

Y : hydrogen atom;

35 Z : = O;

R¹, R², R³ : hydrogen atom

c)

X : -CH₂OR⁷

Y : hydrogen atom;

40 Z : = O;

R¹, R², R⁷ : independently hydrogen atom or WCO-, wherein W represents an alkyl, a substituted alkyl, an aryl, a substituted aryl, an aralkyl or a substituted aralkyl group (provided that all are not hydrogen atom simultaneously);R³ : hydrogen atom;

45 or,

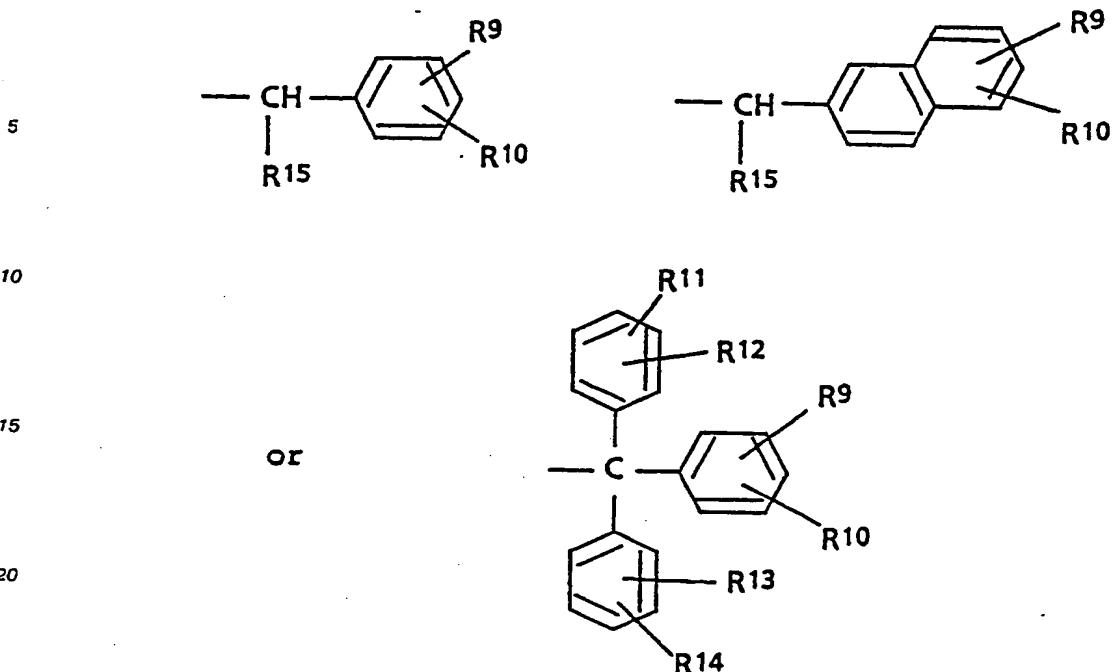
d)

X : -CH₂OR⁸;

Y : hydrogen atom;

Z : = O

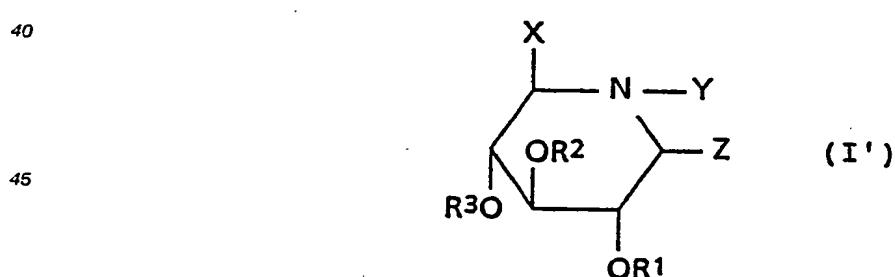
50 R¹, R³ : independently hydrogen atom or an acyl group;R², R⁸ : independently hydrogen atom,



25 R⁹ - R¹⁴ : independently hydrogen atom, a halogen atom, a lower alkyl, a lower alkoxy, a halogenomethyl, carboxyl, a carboxyalkyl, a carboalkoxyalkyl, phenyl or nitro group
 R¹⁵ : a lower alkyl or



2. A compound represented by formula (I'):



50 In formula (I'):
 a)
 X : -CH₂OR⁷;
 Y : hydrogen atom;
 55 Z : = O;
 R¹, R², R⁷ : independently hydrogen atom or WCO-, wherein W represents an alkyl, a substituted alkyl, an aryl, a substituted aryl, an aralkyl or a substituted aralkyl group (provided that all are not hydrogen atom

simultaneously);

R³ : hydrogen atom;

or,

b)

5 X : CH₂OR⁸;

Y : hydrogen atom;

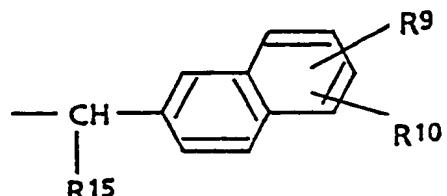
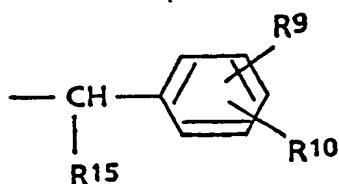
Z : = O

R¹, R³ : hydrogen atom or an acyl group;

R², R⁸ : independently hydrogen atom,

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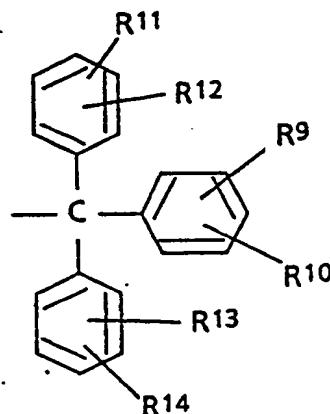


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25

or

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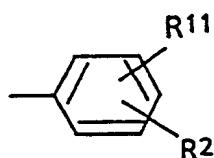


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R⁹ - R¹⁴ : independently hydrogen atom, a halogen atom, a lower alkyl, a lower alkoxy, a halogenomethyl, carboxyl, a carboxyalkyl, a carboalkoxyalkyl, phenyl or nitro group

R¹⁵ : a lower alkyl or

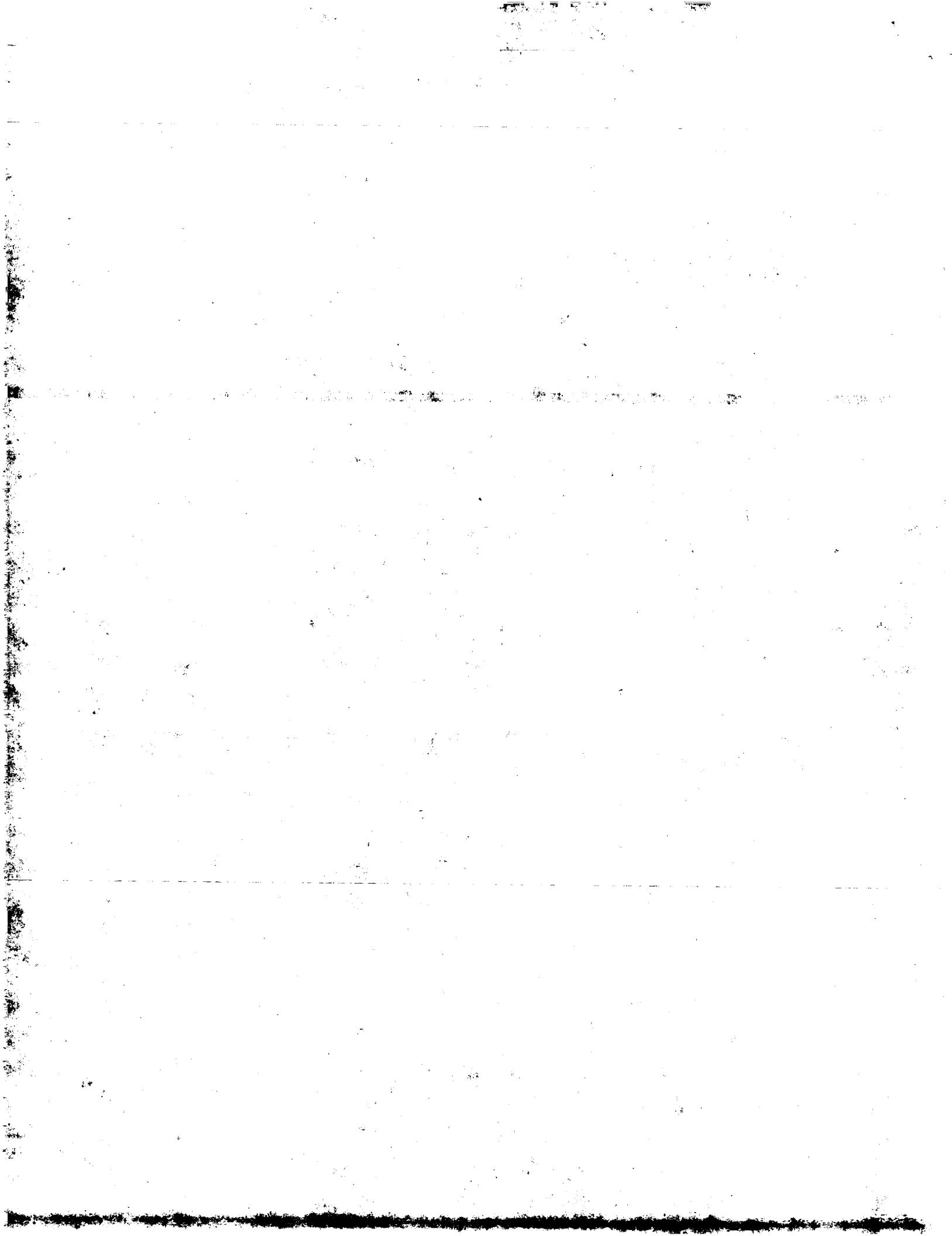
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㉜ Cancer cell metastasis inhibitors.

EP 0 328 111 A3

㉝ Sugar lactams of formula (I) such as N-(3-phenylpropyl)-1-deoxynojirimycin, 1-deoxy-ynojirimycin, D-glucaro- δ -lactam, 6-O-triphenylmethyl-D-gluco- δ -lactam, etc. markedly inhibit metastasis of cancer cells. Sugar lactam derivatives of formula (I') are novel compounds.



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EUROPEAN SEARCH REPORT

Application Number

EP 89 10 2252

DOCUMENTS CONSIDERED TO BE RELEVANT									
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl. 4)						
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<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 34%;">Examiner</td> </tr> <tr> <td>THE HAGUE</td> <td>30-12-1992</td> <td>THEUNS H.G.</td> </tr> </table>				Place of search	Date of completion of the search	Examiner	THE HAGUE	30-12-1992	THEUNS H.G.
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Page 2

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EP 89 10 2252

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X,P	J. CANCER RES. CLIN. ONCOL., vol. 114, no. 2, April 1988, pages 217-220; G. PULVERER et al.: "Glycoprotein modifications of sarcoma L-1 tumor cells by tunicamycin, swainsonine, bromoconduritol or 1-desoxynojirimycin treatment inhibits their metastatic lung colonization in Balb/c-mice" * Entire document * ---	1	
T	JOURNAL OF CELL SCIENCE, vol. 95, no. 2, 1990, pages 279-286, The Co. of Biologists Ltd, GB; E.A. BRUYNEEL et al.: "Effect of glycosylation inhibitors on N-glycosylpeptides and on invasion of malignant mouse MO4 cells in vitro" * Page 280, left-hand column, lines 4-11 * ---	1	
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X,D	GB-A-2 020 278 (NIPPON SHINYAKU CO., LTD) * Entire document * & JP-A-55 009 051 ---	1	
The present search report has been drawn up for all claims			
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THE HAGUE	30-12-1992	THEUNS H.G.	
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Page 3

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EP 89 10 2252

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E	EP-A-0 345 104 (MERRELL DOW PHARMACEUTICALS INC.) * Page 3 * -----	2	TECHNICAL FIELDS SEARCHED (Int. Cl.4)
<p>The present search report has been drawn up for all claims</p>			
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